Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-21 (Cancelled)

22. (Currently amended) A method for modulating G-protein mediated signal transduction comprising:

providing a cell having a disturbed G-protein mediated signal transduction and a receptor tyrosine kinase capable of activation by G-protein mediated signal transduction;

contacting the cell with a compound affecting ~~an extracellular~~ a G protein or G protein coupled receptor initiated extracellular signal pathway resulting in an activation of the receptor tyrosine kinase and thereby modulating the receptor tyrosine kinase activation by G-protein-mediated signal transduction.

23. (Previously presented) The method according to claim 22, wherein said receptor tyrosine kinase is epidermal growth factor receptor (EGFR).

24. (Previously presented) The method according to claim 22, wherein said compound affecting an extracellular G protein or G protein coupled receptor initiated signal pathway affects (I) a proteinase cleaving a precursor of a ligand

25. (Previously presented) The method according to claim 24, wherein the compound affects the proteinase by directly stimulating or inhibiting proteinase activity.

26. (Previously presented)The method according to claim 24, wherein said precursor of a ligand is a membrane associated molecule.

27. (Previously presented) The method according to claim 26, wherein said precursor of a ligand for the receptor tyrosine kinase is proheparin-epidermal growth factor (proHB-EGF) and said receptor tyrosine kinase is EGFR.

28. (Previously presented)The method according to claim 24, wherein said proteinase is a membrane-associated proteinase.

29. (Previously presented) The method according to claim 24, wherein said proteinase is a metalloproteinase.

30. (Previously presented) The method according to claim 29, wherein said metalloproteinase is a zinc-dependent proteinase.

31. (Previously presented) The method according to claim 24, wherein said proteinase activity is inhibited by batimastat.

32. (Currently amended) The method according to claim 22, ~~wherein said compound affects a cell which is different from the cell containing the receptor tyrosine kinase~~ further comprising a second cell which is different from the cell containing the receptor tyrosine kinase, wherein said compound affects said second cell.

33. (Previously presented) The method according to claim 22, wherein said receptor tyrosine kinase is selected from the group consisting of EGFR, HER-2, HER-3, HER-4, TNF receptor 1, TNF receptor 2, CD 30 AND IL-6 receptor.

34. (Previously presented) The method according to claim 22, wherein said receptor tyrosine kinase is selected from the group consisting of EGFR and other members of the EGFR family.

35. (Previously presented) A method for identifying compounds for modulating G-protein mediated signal transduction, comprising contacting a cell containing a receptor tyrosine kinase capable of activation by G-protein mediated signal transduction with a test compound suspected of being a modulator of a proteinase or a precursor of a ligand of the receptor tyrosine kinase, and evaluating G-protein mediated receptor tyrosine kinase activation upon exposure of the cell to said test compound.

36. (Currently amended) A method for modulating a G-protein mediated signal transduction

comprising:

providing a cell having a disturbed G-protein mediated signal transduction and a receptor tyrosine kinase capable of activation by G-protein mediated signal transduction, wherein said receptor tyrosine kinase is selected from the group consisting of EGFR and other members of the EGFR family, said cell comprising an extracellular domain and having a G-protein mediated signal transduction pathway wherein one or more tyrosine residues are phosphorylated based on the activation of signal transduction pathway, the extracellular domain of said receptor is capable of binding to its receptor ligand, and said ligand is generated from a precursor of said ligand by a proteinase-dependent cleavage;

contacting said cell with a compound affecting ~~an extracellular~~ a G protein or G protein coupled receptor initiated extracellular signal pathway resulting in the activation of the receptor tyrosine kinase and thereby modulating the receptor tyrosine kinase activation by G-protein mediated signal transduction.

REMARKS

In the Office Action dated May 20, 2003, claims 22-36, in the above-identified U.S. patent application were rejected. Reconsideration of the rejections is respectfully requested in view of the above amendments and the following remarks.

Claim 27 was objected to as being of improper dependent form for not further limiting the receptor kinase in claim 23. Applicants respectfully point out that claim 27 does not depend from claim 23. Claim 27 indirectly depends from claim 22 and does further limit claims 22, 24 and 26 from which it depends. In view of this, applicants request that this rejection be withdrawn.

Claims 22-34 and 36 were rejected under 35 USC §112, second paragraph. Claims 22 and 36 were rejected due to the language "extracellular G protein". Claims 22 and 36 have been amended to clarify that the term "extracellular" refers to the signal pathway not the G protein or G protein coupled receptor. Claim 32 was found indefinite regarding a cell which is different from the cell containing the receptor tyrosine kinase. Claim 32 has been amended to clarify that there is a second cell which is different from the cell containing the receptor tyrosine kinase. In view of the above amendments, applicants request that these rejections be withdrawn.

Claims 22-36 were rejected under 35 USC §102(a) or alternatively under 35 USC §103(a) as anticipated by or as obvious over Dong et al. Applicants respectfully point out that at the time of the present invention, it was believed that the correlation between G protein activation and the activation of tyrosine

phosphorylation of EGFR was mediated by an intracellular pathway. Thus, one skilled in the art would not have expected batimastat, which acts on an extracellular pathway of EGFR, to be capable of modulating a G protein mediated signal transduction. As stated on page 6 of the office action, Dong does not directly show that their method is related to modulation of G-protein mediated signal transduction. In addition, Dong does not indicate that the cells he used have a disturbed G-protein mediated signal transduction as required in the present claims. Applicants contend that in view of the knowledge in the art, one skilled in the art would not have been motivated to modify Dong's method to modulate G protein mediated signal transduction in a cell having a disturbed G-protein mediated signal transduction.

In addition, at the bottom of page 5, under point (2), the office action states: "*since it is known that reduction of tyrosine phosphorylation of a receptor is correlated to activation of G protein, batimastat used in the method of Dong et al. also modulate G protein mediated signal transduction.*" Applicants point out that this statement is both incomplete and incorrect. A correlation of G protein activation and the activation of tyrosine phosphorylation of EGFR was indeed known in the art (e.g. Daub et al). It was assumed, however, that this correlation is mediated by an intracellular pathway. Thus, prior to the present invention, one skilled in the art could not reasonably have expected that batimastat as used in the method of Dong et al. (which acts on an extracellular activation pathway of EGFR) would be capable of modulating a G protein mediated signal transduction. The activation of receptor tyrosine kinases such EGFR can be effected via a

plurality of different pathways. As explained in detail below, a number of different stimuli were known, in addition to the activation of G proteins, which were correlated with EGFR tyrosine phosphorylation at the time of the Dong et al publication. For example, it had been reported at the time of Dong et al. that the stimulation of cytokine receptors leads to tyrosine phosphorylation of the EGF receptor (Yamaguchi et al., 1997, Tyrosine phosphorylation of the EGF receptor by the kinase JAK2 is induced by growth hormone. Nature 390: 91-96). However, in contrast to the mechanism observed by Dong et al., this pathway involves the JAK family of intracellular non-receptor tyrosine kinases and requires the intracellular adaptor-docking function of the EGF receptor (Yamaguchi et al., 1997).

It was also known at the time of Dong et al. that members of the integrin family of cell surface receptors can modulate EGFR tyrosine phosphorylation in order to generate further cellular responses (Moro et al., 1998, Integrins induced activation of the EGF receptor: role in MAP kinase induced and adhesion-dependent cell survival. EMBO J 17: 6622-6632). However, again in contrast to the mechanism observed by Dong et al., this pathway is independent of EGFR ligands and involves cell adhesion dependent interaction of integrins with the EGFR (Moro et al., 1998).

Furthermore, it was well documented at the time of Dong et al. that a number of exogenous stress stimuli, both physical and chemical, initiate signal transduction pathways in cells that are part of stress responses. In particular, UV radiation had been reported to promote enhanced tyrosine phosphorylation of the

EGF receptor (Warmuth et al., 1994. Ultraviolet radiation induces phosphorylation of the epidermal growth factor receptor. Cancer Res. 54: 374-376). However, as UV activates v-erbB, an oncogenic isoform of the chicken EGF-receptor that lacks a ligand-binding domain, the mechanism of UV-induced EGFR activation occurs ligand-independently (Knebel et al 1996, Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. EMBO J. 15: 5314-5325). In fact, activation of the EGFR is indirect through inactivation of phosphotyrosine phosphatases (Knebel et al., 1996).

Finally, it had been demonstrated at the time of Dong et al. that the phorbol ester TPA (12-O-tetradecanoylphorbol-13-acetate) promotes EGFR tyrosine phosphorylation via its intracellular receptor protein kinase C (Xian, W. et a., 1995, Activation of the epidermal growth factor receptor by skin tumor promoters and in skin tumor from SENCAR mice, Cell Growth & Differentiation 6: 1447-1455; Emkey and Kahn, 1997, Cross-talk between phorbol ester-mediated signaling and tyrosine proto-oncogenenes, J. Bio. Chem. 272: 31172-31181). The present application (see also Prenzel et al., 1999) discloses that this TPA-induced transactivation of the EGF receptor occurs via metalloproteases and HB-EGF but is clearly distinct from GPCR mediated EGFR receptor activation (see Figures 2c and 3a of the present application).

In view of the above discussion, applicants contend that it cannot be assumed from the mere correlation of the effect of the compound according to Dong et al. on the proteolytic release of EGFR ligands that it modulates GPCR

mediated EGFR tyrosine phosphorylation, particularly since there were non-GPCR mediated EGFR tyrosine phosphorylation pathways which are also inhibited by batimastat. At the time of Dong et al. it was commonly believed that GPCR stimulation of EGFR activation itself did not involve proteolytic cleavage of ligand precursors. For example, at the time of Dong et al. it was generally acknowledged that the mechanism by which GPCRs modulate tyrosine phosphorylation of the EGF receptor is centered on the mediation by the non-receptor tyrosine kinase c-Src, which was reported to be coupled to nearly all GPCRs that lead to EGF receptor phosphorylation (reviewed in Thomas and Brugge, 1997, Cellular functions regulated by Src family kinases. Annu. Cell Dev. Biol. 15: 513-609). Over expression of either a dominant-negative Src construct or Csk, a regulatory kinase that inhibits Src function, decreases EGF receptor tyrosine phosphorylation provoked by activation of LPA or α2 adrenergic receptors (Luttrell et al., 1997, Gby subunits mediated Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for G protein-coupled receptor mediated Ras activation. J. Biol. Chem. 272:4637-4644). The mediator role of Src was suggested to be direct in that Src is able to associate with and phosphorylate the EGF receptor *in vivo* and *in vitro* (Thomas and Brugge, 1997). This mechanism would predict the existence of Scr-EGF receptor complexes provoked by activation of GPCR. The evidence for this was shown by the demonstrations that angiotensin II (Eguchi et al., 1998, Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular

smooth muscle cells, J. Biol. Chem. 273: 8890-8896) or LPA (Luttrell et al., 1997) rapidly increases the amount of Src coprecipitated with EGF receptors.

Furthermore, calcium influx was reported to be sufficient to trigger EGFR tyrosine kinase phosphorylation and MAP kinase activation in PC12 cells (Rosen and Greenberg, 1996, Stimulation of growth factor receptor signal transduction by activation of voltage-sensitive calcium channels. Proc. Natl. Acd. Sci. USA 93: 1113-1118). This had been extended subsequently since several findings had demonstrated Ca^{2+} to be necessary for EGFR-transactivation induced by GPCR-ligands (Eguchi et al., 1998; Eguchi et al., 1999, Involvement of PYK2 in angiotensin II signaling of vascular smooth muscle cells, Hypertension 33: 201-206; Soltoff 1998, Related adhesion focal tyrosine kinase and the epidermal growth factor receptor mediate the stimulation of mitogen-activated protein kinase by the G-protein-coupled P-2Y2 receptor, J. Biol. Chem 273: 23110-23117). Due to this critical function of Ca^{2+}, Ca-regulated FAK family kinase PYK2 was discussed as a mediator of EGFR transactivation in the signaling elicited by GPCR ligands upstream of the EGFR signal (Eguchi, 1999; Soltoff 1998).

In view of the above discussion, the correlation of GPCR-induction of EGFR activation via a pathway comprising extracellular elements is not suggested or disclosed by Dong et al. Thus one skilled in the art would not be motivated to modify Dong et al to contact a cell having a disturbed G-protein mediated signal transduction with a compound which affects an extracellular signal pathway as required by the present claims and applicants request that this

rejection be withdrawn.

Applicants respectfully submit that all of claims 22-36 are now in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

<table>
<tr><td colspan="6">RESPECTFULLY SUBMITTED,</td></tr>
<tr><td>NAME AND REG. NUMBER</td><td colspan="5">Monica Chin Kitts, Registration No.: 36,105</td></tr>
<tr><td>SIGNATURE</td><td colspan="3">  </td><td>DATE</td><td>8/20/03</td></tr>
<tr><td>ADDRESS</td><td colspan="5">Rothwell, Figg, Ernst & Manbeck
Suite 800, 1425 K Street, N.W.</td></tr>
<tr><td>CITY</td><td>Washington</td><td>STATE</td><td>D.C.</td><td>ZIP CODE</td><td>20005</td></tr>
<tr><td>COUNTRY</td><td>U.S.A.</td><td>TELEPHONE</td><td>202-783-6040</td><td>FAX</td><td>202-783-6031</td></tr>
</table>